A quantitative assessment of the content of hematopoietic stem cells in mouse and human endosteal-bone marrow: a simple and rapid method for the isolation of mouse central bone marrow.

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Public Summary:
Abstract BACKGROUND: Isolation of bone marrow cells, including hematopoietic stem cells, is a commonly used technique in both the research and clinical settings. A quantitative and qualitative assessment of cell populations isolated from mouse and human bone marrow was undertaken with a focus on the distribution of hematopoietic cells between the central bone marrow (cBM) and endosteal bone marrow (eBM). METHODS: Two approaches to cBM isolation from the hind legs were compared using the C57BL/6J and BALB/cJ strains of laboratory mice. The content of hematopoietic stem cells in eBM was compared to cBM from mice and human fetal bone marrow using flow cytometry. Enzymatic digestion was used to isolate eBM and its effects on antigen expression was evaluated using flow cytometry. Humanized immunodeficient mice were used to evaluate the engraftment of human precursors in the cBM and eBM and the effects of in vivo maturation on the fetal stem cell phenotype were determined. RESULTS: The two methods of mouse cBM isolation yielded similar numbers of cells from the femur, but the faster single-cut method recovered more cells from the tibia. Isolation of eBM increased the yield of mouse and human stem cells. Enzymatic digestion used to isolate eBM did, however, have a detrimental effect on detecting the expression of the human HSC-antigens CD4, CD90 and CD93, whereas CD34, CD38, CD133 and HLA-DR were unaffected. Human fetal HSCs were capable of engrafting the eBM of immunodeficient mice and their pattern of CD13, CD33 and HLA-DR expression partially changed to an adult pattern of expression about 1 year after transplantation. CONCLUSIONS: A simple, rapid and efficient method for the isolation of cBM from the femora and tibiae of mice is detailed. Harvest of tibial cBM yielded about half as many cells as from the femora, representing 6.4 % and 13 %, respectively, of the total cBM of a mouse based on our analysis and a review of the literature. HSC populations were enriched within the eBM and the yield of HSCs from the mouse and human long bones was increased notably by harvest of eBM.

Scientific Abstract:
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